

REVIEW

**The Peculiar Case of the Giants of *Oxytricha bifaria*
(Ciliata, Hypotrichida): a Paradigmatic Example
of Cell Differentiation and Adaptive Strategy**

NICOLA RICCI and ROSALBA BANCHETTI

*Dipartimento di Scienze dell'Ambiente e del Territorio
Via A Volta, 6-56100 PISA, Italy*

INTRODUCTION

In a stage of biological research such as today's, characterized by the extreme specialization of each scientist, in turn due to the need of obtaining ever new information about Life, the quantity of new reports available daily, even within a relatively "restricted" area such as protozoology is so large and evergrowing, that most of us cannot study and master much of the recent knowledge, even in the field of their own specific interest. It is our opinion that every day it becomes more and more practically urgent and culturally necessary for all of us to make all possible attempts to reconsider each of our results in a double perspective, if we want to avoid the danger of creating isolated, limitedly useful pieces of Science. On one hand, we must place them correctly in the wider context of Nature, but try to interpret them in the general light of evolutionary biology on the other. This is the rationale of the present review article, in which the whole story of the giants of *Oxytricha bifaria* will be described overall and then discussed (and the relative implications examined) in its double-faced valence, namely both as an example of cell differentiation and of reversible carnivorousness.

The idea of a review article about nature and biology of the giants of *Oxytricha bifaria* (Ciliata, Hypotrichida) arose when the overall picture of the process became sufficiently exhaustive and self-standing as a consequence of a complex round

of experiments recently carried out [59, 60, 65, 66, 69, 71]: it now lends itself to be rationalized in terms of a general model which, in turn, can be used fruitfully to penetrate further into the general adaptive strategies which shaped the history of life.

Protozoa are as unique and peculiar as they are precious, for approaching many of the most fundamental problems of biology with ever new investigation tools and a fresh mind. What makes them unique among the other organisms is their double sided nature: each single protozoon is indeed a perfect eukaryotic cell ("a physiological unit") and a complete organism ("a selective unit") at the same time. This character of theirs enables us to pass from the cellular to the organismic-adaptive level directly, without approximations. Protozoa, moreover, were the first eukaryotes to appear in the primeval Ocean: they are very ancient organisms, 2.2 billion years old [47]. This trait of Protozoa is a highly relevant one, once we consider that they reached first, the eukaryotic organization, exploiting then all its potentialities and realizing the widest range of variations on the theme "eukaryotic cell", adapting their morphology and physiology to match very different environmental challenges. We could say that Protozoa reach the highest peaks of complexity at the cellular level, in much the same way as metazoa do at that of the organismic level [3]. This consideration, then, should urge us to make a sort of "Copernican revolution" in Biology, leading us to recognize their primigenial nature and to use them more correctly than to date. From this point of view,

indeed, it was Protozoa which first found the correct solutions of most general adaptive problems (regulation of differentiative processes, exchange of information through cellular interactions, cell locomotion as behaviour capable of reaching optimal conditions of environmental factors), so that we should learn to study and to understand them according to the characters of their own biology, instead of continuing to consider them as just "simple metazoa" or even as sort of "simple" free-living lymphocytes, neurons, etc. This thought will prove to be quite a relevant one in section III, when the cell differentiation aspects of the process object of this article will be discussed.

In our opinion Protozoa are among the best biological material for a wide interdisciplinary investigation enabling us to collect information which can be compared and integrated directly and immediately. The clearest tendency illustrating this theory of ours is the eco-ethological approach recently proposed for protozoa by Ricci [57]: he tends to link the study of their behaviour [54], with that of their ecology [16] in an attempt to comprehend their general adaptive philosophy. The first line of research has its roots in the studies of cell locomotion [33, 34], but draws new strength and perspectives when the electrophysiological studies [40, 41] are brought in [56]. The second half of this study, namely the ecological one was suggested by Fenchel in his masterpiece [16], and it led to a new understanding of the importance of the protozoa's microbial loop in aquatic environments, of their totally anti-intuitive world [53, 62, 63, 64], of their usefulness in monitoring the environmental conditions [7, 17, 55].

This example shows how powerfully these rather disregarded organisms can be investigated by means of widely different technical approaches to achieve very useful results in widely different fields of Biology: what we would like to do in the present paper is to describe the complete picture of the morpho-functional steps leading a normal *Oxytricha bifaria* to differentiate into a gigantic organism, to discuss then this story in the more general context of "cell differentiation" problematics, on one hand, and in that of the different secondary consumer adaptive strategies, on the other.

I The giant of *O. bifaria*: a multi-step adaptation

The data henceforth described as a single body of information, were published in six papers [59, 60, 65, 66, 69, 71] as the results of partial rounds of experiments carried out from 1982 to 1990, in which all the technical and methodological details can be found concerning both the culturing of *O. bifaria* and the various experimental approaches. The steps in the story of the giant in *O. bifaria* will be discussed in series, putting together all the available information, each indicated by one of the above numbers, to facilitate bibliographic consultation.

O. bifaria is a freshwater hypotrich ciliate [8], with a typical ellipsoidal body ($\sim 110 \times 60 \mu\text{m}$) differentiated into a convex dorsal surface and into a more or less planar ventral one, where all the composed ciliary organelles are placed: they can be distinguished into (a) somatic locomotory organelles (namely the fronto-ventral, transverse and marginal cirri) and (b) peristomial organelles (namely the anterior-left Adoral Zone Membranelles, AZM, and the mid-ventral Undulating Membranelles (UM) (Fig. 1). The nuclear apparatus is formed by one macronucleus divided into two pieces and by two micronuclei, each close to either macronuclear envelope. *O. bifaria* lives in freshwater canals, springs, creeks, rivers, ponds, lakes, typically feeding on bacteria: its cell cycle [12, 58] usually lasts about 7–8 hr at about 20°C. As to its life cycle it is characterized by quite a long immaturity period (~ 180 binary fissions, Ricci & Cetera, unpublished results), following a sexual phenomenon (conjugation), by a maturity period (which lasts no longer than 2–3 years, under laboratory conditions, Ricci & Banchetti, unpublished results) and by a rather short senescence, (progressive loss of the mating competence) un-failingly leading to the death of the clone. The general description of *O. bifaria*'s biology cannot be considered complete, without taking into account the nature of its habitat; in the natural environment where we find this species, indeed, we measured very different conditions at different times as to temperature (-2°C to 36°C), to pH (5.7–7.5) and dissolved oxygen (3.5 mg solved oxygen/ml), not to speak of the water itself,

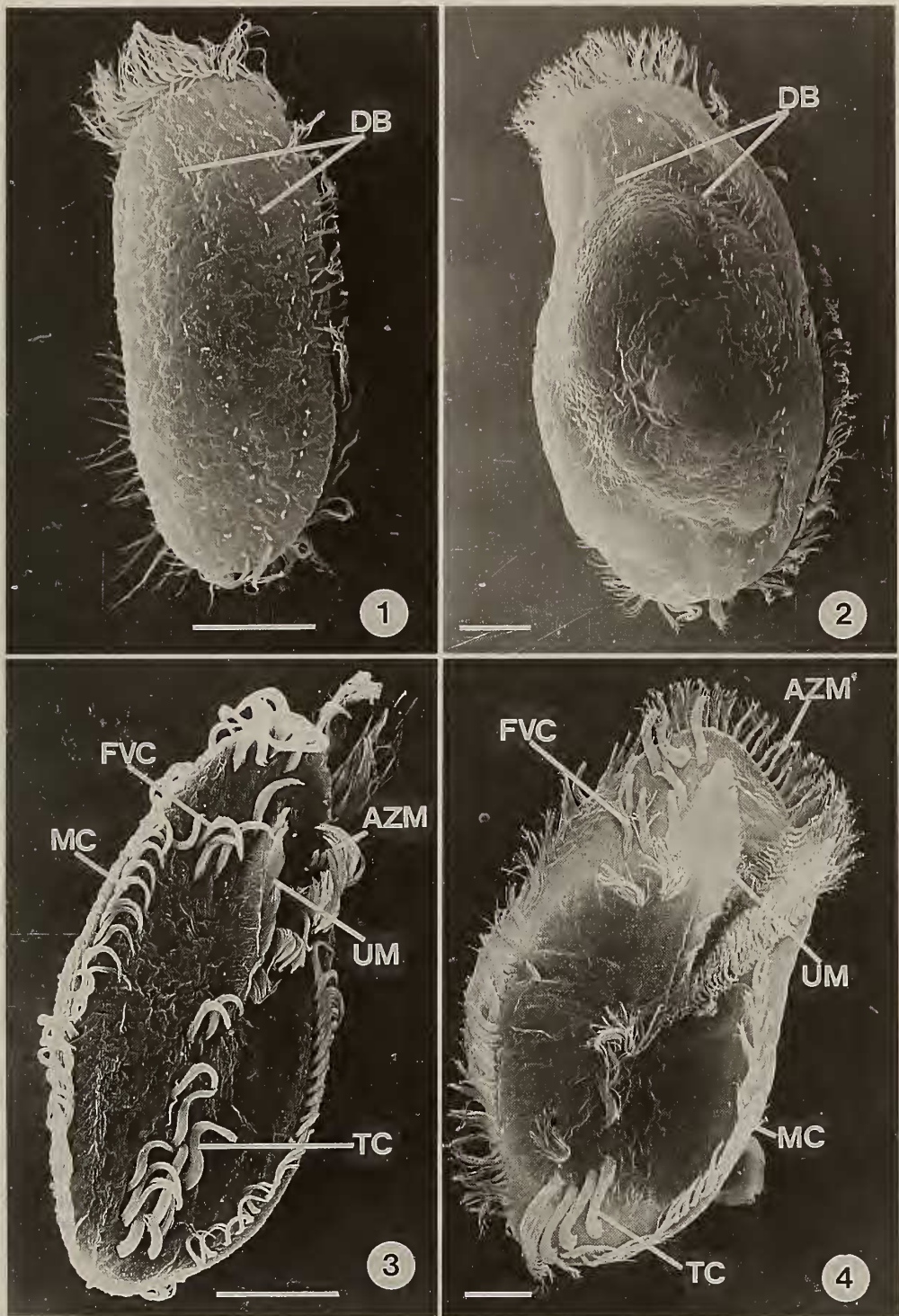


FIG. 1. The normal *Oxytricha bifaria*, in dorsal (1) and ventral (3) views; the giant of the same species, in dorsal (2) and ventral (4) views. AZM: Adoral Zone Membranelles; UM: Undulating Membranelles; MC: Marginal Cirri; FVC: Fronto-Ventral Cirri; TC: Transverse Cirri; DB: dorsal bristles. The bar represents 50 μm.

which, quite frequently in the summer, can disappear completely (Ricci & Banchetti, unpublished results). We could say that the environment of *O. bifaria* (that of inland, often impermanent waters) is characterized by the widest (and wildest) variations of the physical, chemical and biological parameters describing it: in agreement with such an extreme environmental variability, the species we have been studying since 1972, shows a similarly extreme adaptability, being capable of changing dramatically both its shape and its physiology as it is required to withstand the challenges periodically met in such an environment. Although more than 95% of its life span is spent in the normal morpho-physiological state, three other states are possible and actually found in our samples collected in nature in different periods of the year (Fig. 2).

The conjugating pair is a peculiar morpho-functional state formed by two oxytrichas united side by side, which carry on their sexual processes,

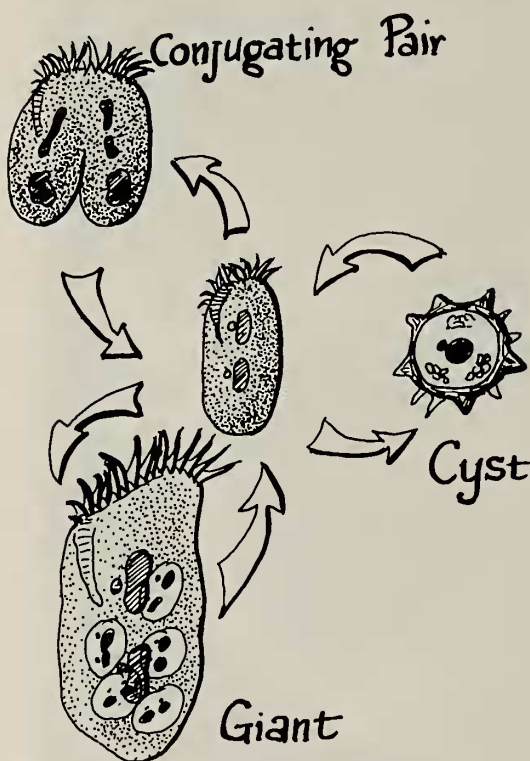


FIG. 2. *O. bifaria* spends ~95% of its lifetime as normal organisms, capable of differentiating conjugating pairs, cysts and giants, according to the internal/external conditions of the system.

through a complex cascade reaction: (a) two-step cell interactions leading to (b) cell membrane fusion, (c) trigger of micronuclear meiosis, actual (d) meiotic divisions of micronuclei, (e) exchange of pronuclei (=cross fertilization), (f) separation of the partners [52]. Such a complex sexual process mediates the rejuvenation of the population: it can be adaptively interpreted as the periodical solution (triggered by proper environmental conditions) found by the species to the problem of overcoming its main biological bottleneck, namely the absolute need of producing genetic recombinants and of fighting progressive ageing.

Cysts represent the second possibility *O. bifaria* evolved to match the challenges periodically met in its widely changing environment: they represent a sort of life-boat for the species when severe, prolonged environmental stresses (drought in summer, for instance) affect natural populations. The dramatic morpho-physiological changes a cell undergoes to produce the sophisticated structure that a cyst is, were studied mainly from ultrastructural [75], cytochemical [68] and adaptive *s.l.* points of view [67].

On the basis of the above integrated results concerning the nature and story of both pairs and cysts, a thorough, specific giant formation was then undertaken.

A Normal populations

This is the phase preceding the onset of any specific induction of the phenomenon "giant-formation".

O. bifaria is a species whose natural populations can produce gigantic organisms: only 1–2% of the strains collected in nature, however, show such a capability. A certain strain, which proves to be capable of differentiating giants, under proper conditions, tends to maintain such a trait steadily in time.

Many strains have been collected and stocked in Lab cultures and each clone has its own giant producing potentiality, which can be measured by two parameters: (i) number of giants produced and (ii) time lag before the appearance of the first giant. According to these two traits, the clones we used in the last 8 years can be ordered as it follows:

C9>S9>S6, C9 being the strain producing the largest number of giants in the shortest time. No exhaustive theory has been proposed so far to account for the nature of the character "giant formation": no clear-cut genetic inheritance of this trait has yet demonstrated, for any clone, isolated and cultured in our Lab. Only very few cells can produce giants *ex-novo* even in the richest population of *O. bifaria* belonging to a giant-producing strain: these few cells have such a capability only for a relatively short time, being then substituted by other individuals, in turn only temporarily predisposed for such an adaptive task (Ricci and Pelamatti, unpublished results). It has been found, indeed, that although hundreds of giants can be observed in the experimental populations, by far the largest part of their number is produced through binary fission of preexisting giants, while only very few (2–3 per day) are differentiated *ex-novo* from normal individuals, even in cultures as dense as 1600 cells/ml. While the problem of the nature of this labile predisposition to produce giants seems today far from being accounted for, both at clonal and at individual level, it might be simpler to answer to the question "why can so few, and so frequently changing oxytrichas differentiate a giant?" In our opinion, a certain degree of loss of fitness *s.l.* is to be expected for the "ready-to-differentiate" organisms, while a clear advantage is evident for the gigantic forms. If this is true, the labile determination of only few oxytrichas to differentiate giants might be considered as the best solution to limit the reduction of fitness within the population (due to the acquisition of such a potential state which only very rarely actually leads to the advantageous gigantic form) without missing the adaptive convenience brought to the population by the occurrence of such a differentiated gigantic state. The temporal lability of the determination to produce giants could have been acquired to avoid the risks of a progressive reduction of genetic variability in time, if only a few cells (and only they) should be destined to become giants: all the possible genomic combinations of a certain genetic pool, indeed, have the same, although small, probability of undergoing the gigantic adventure.

This general hypothesis seems to us supported and strengthened by the finding that, as the indi-

viduals of a certain population are periodically determined to undergo gigantic differentiation, there is no convenience for any population to have a large number of organisms predisposed to cell differentiation: the need of producing a large population of giants, whenever a proper rich *pabulum* should exist, is matched by the cell-binary-fission-mechanism, a fairly convenient and fast asexual reproductive process.

Terminological forward: (i) the time lag between the onset of the conditions suitable for the formation of giants and the actual formation of the first giant has been defined "induction period" and indicated " Δt FG"; (ii) the induction period is formed by an "activation period", followed by the successive "predation", namely the actual feeding of activated cells on the potential preys: (iii) within the activation, two successive periods have been distinguished: the "Early-Activation Window" (= EAW, roughly corresponding to the first third of Δt FG) and the "Late-Activation Window" (= LAW, occurring during the second third of the Δt FG): the last third of the Δt FG corresponds to predation. All these concepts are illustrated in Fig. 3.

B The Early Activation Window (EAW)

Giants of *O. bifaria* form whenever favourable conditions are created [1], namely whenever an overcrowded population is obtained. Different strains have different thresholds for the occurrence of the phenomenon. "Threshold" can be defined as the lowest cell density capable of producing a First Giant in that strain within 12 hr from the onset of the overcrowding conditions: as an example #C9 had a threshold of 340 cells/ml, while #S9 and #S6 had thresholds of 390 and 500 cells/ml, respectively (Ricci & Cifarelli, unpublished results).

Starvation, on the contrary, has been shown to play only a minor, disturbing role in giant formation. A clearcut, statistically significant, positive linear correlation has been found to occur between the cell density of an overthreshold population and the number of giants formed in time: only beyond extreme values of cell densities (>40,000 cells/ml for #C9) this tendency shows a clear reversal, very likely due to the strong disturbance of the

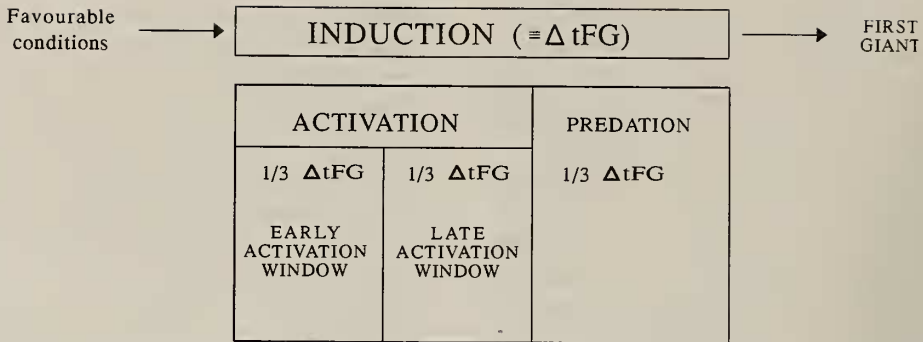


FIG. 3. The temporal succession and the various relative correspondances of the phases of the process leading on *O. bifaria* from its normal state to the production of the first giant.

locomotory behaviour of the potential giants, in turn induced by the continuous, unavoidable cell-against-cell bumps [66].

It has been found also that there is a clear, significant, negative, linear correlation between the number of cells/ml and the Induction Period ($\Delta t FG$): optimizing the number of cells/ml, their biological state and growing conditions, it has been found that a minimum, non reducible, $\Delta t FG$ exists and that it lasts approximately 45 min [65]: thus, we can say that the higher the cell density of the overthreshold population, the shorter the induction period, the larger the total number of the giants produced.

The biological phenomenon controlling the beginning of the story of the differentiation of giants in *O. bifaria* (and guiding its development later on) is a really interesting contact-dependent cellular interaction, demonstrated by using Con-A as a specific inhibitor of the process [59]: no soluble factor plays any role, as shown by the lack of the slightest effects of both the Cell Free Fluid of giant-producing populations and the perfusion microchamber experiments, using top quality cultures [59]. The adaptive reasons of this choice (the choice of using short range signals to trigger the differentiation of giants rather than long range soluble factors, as happens for the conjugation of the same *O. bifaria*, [52]) seems to us to reside in the nature itself of the giants of *O. bifaria*, which are true, opportunistic, all-devouring carnivores, rather than cannibals: direct contact seems to us more effective than soluble factors in informing a potential giant about the number of the preys

living in the water volume surrounding it, namely about the possible convenience of differentiating into an actual giant.

Several things must be said to characterize the kind of cell contacts required by this differentiation. First of all they must occur between normally behaving cells: neither frozen-thawed nor mildly $K_2Cr_2O_7$ fixed ciliates ever induce any kind of activation [65]: according to these results we can expect a mechanism very likely relying on a certain activation energy which enables *O. bifaria* to distinguish living (=food) from inert (=no food) objects and, therefore, to avoid senseless, quite expensive differentiations of giants [65]. Thus, it seems to us more appropriate to speak of active contacts, or bumps, which must occur among different cells, to trigger the process, rather than of simple, cellular contacts. By the way, quite a similar recognition mechanism seems to occur also in the story of *Litonotus*, a specific, a very efficient predator feeding on *Euplotes* (Ricci & Verni in prep.).

A second trait characterizes the bumps activating induction: they are not species-specific bumps. It has been demonstrated that many different species of ciliated protozoa can bump fruitfully against an oxytricha, activating it to differentiate into a giant, provided that it has such a potentiality. In our experiments *Paramecium aurelia* perfectly succeeded in activating *O. bifaria*, the only difference being that the threshold for giant formation induction was a little higher than with conspecific individuals: $\sim 1,000$ paramecia/ml are required to trigger the process, while only 340

oxytrichas/ml are sufficient for the same effect [59]. The adaptive meaning of the giant of *O. bifaria* is clearly demonstrated by such a finding: we cannot speak of cannibals [66] any more, while we have to look at this heteromorphic state as a true carnivorous gigantic form [59]. The basic nature of the giant of *O. bifaria* is well described by several elements characterizing the differentiation: (a) the choice of direct interactions: (b) their non species-specificity; (c) the prompt answer (Δt FG $\sim 40'$) induced by favourable conditions. This way of understanding the biological significance of the giants of *O. bifaria* is of the highest relevance to interpret the giant itself properly in the context of this species' adaptive biology: not any more a form by which the species escapes environmental stress conditions feeding the few at the expenses of the many conspecific organisms, but a form through which it opportunistically shifts from its normal diet (bacteria) to a new, temporarily richer *pabulum* (other ciliates). In so doing, *O. bifaria* changes its trophic niche, shifting from its normal one (primary consumer) to a new one, that of a secondary consumer. The general interpretation is very likely to be found in the attempt of this bacterivorous species to prolong opportunistically its existence in a certain spatial spot growing at first at the expenses of bacteria ("minimum", ever present food), and later feeding on the ciliated population supported by the bacteria themselves: by producing the giants, *O. bifaria* reaches at least four very convenient goals: (a) it reduces the intraspecific competition for bacterial food; (b) it reproduces at the expenses of different species; (c) by predated the bacterivorous species it reduces the interspecific competition for bacterial food; (d) it survives in the first favourable micropatch for a longer period, the peak of primary consumers being reached later than that of bacteria [16].

One surprising thing must be added with regard to the nature of the bumps triggering the giant formation: they can occur even with organisms not lending themselves as preys [65]! *Blepharisma japonicum* ($\sim 300 \mu\text{m}$ long) induces giants in *O. bifaria* populations, whose cells are activated, although having no possibility of predated those large organisms! This kind of experiment is the only one so far enabling us to separate the inducing

stimuli (cell-cell bumps) from the presence of preys: this possibility is a truly important one for further investigations about the nature of the molecular aspects of this part of the story. The finding that organisms not suitable as preys induce the giant formation demonstrated that "activation" truly exists as a biological step in the process leading to giant formation. The occurrence of these peculiar bumps in the first third of the Δt FG is a prerequisite for the process to continue [60]: thus, the objective existence of an Early Activation Window (=namely a short period during which, and only during which, something conditioning the occurrence itself of the whole process has to happen!) has been shown clearly by simple, successive dilutions of experimental populations. The conclusion to draw is therefore that, if proper bumps occur at the right moment, they cannot but trigger the physiological steps of the next phase, the Late Activation Window [60].

Before concluding this chapter about the nature of the phenomena leading to giant formation in *O. bifaria*, the so called "Labile Memory Counter" (LMC) working hypothesis [65] must be briefly described and discussed: the LMC is supposed to be a cellular device somehow capable of counting the cell bumps activating the cortical keys, of recording them for a while (labile memory) and of adding the new contacts to the total: when the sum reaches a certain threshold value (within a certain period of time?) the initial processes of activation period are triggered. Although nothing can be proposed about the basic nature of the LMC, it accounts for several of the observations so far made about the giant differentiation story: the different, strain specific "thresholds" for instance, may be explained in terms of (a) different strain specific number of cortical activating keys, (b) different strain specific number of bumps necessary for the activation, (c) different forgetting velocities, (d) different key activation energy.

C The Late Activation Window (LAW)

The second half of the Activation (roughly corresponding to the second third of the induction period) has been found to be characterized by the occurrence of a specific protein synthesis triggered by the LMC when the threshold is reached. While

the use of Act-D had demonstrated the relevance of the role played by some protein synthesis, specific low dosage-short term pulses of cycloheximide demonstrated the temporal occurrence of that synthesis [60]. It was also found that higher dosages of Chx induce longer Δt FG and smaller number of giants formed: this seems to indicate that the Chx action is a fairly specific one, inducing just quantitative, absolutely physiological effects. It will be of great interest to ascertain the nature of the specific protein synthesized during the LAW, also in attempting to understand the nature of its action, which, in turn, very likely represents the basic meaning of LAW.

D Predation

Both the EAW and the LAW proved to be necessary, but not sufficient steps for the differentiation of giants of *O. bifaria*: the actual engulfment of several/many preys is necessary (as shown by the induction by *Blepharisma* [65]) to make the potentialities triggered by the activation real, namely to change the gross cell morphology, thus producing the First Giant. Activated, morphologically normal oxytrichas feed successfully on casually encountered preys because of their peculiar behaviour. Although specific ethological studies of the problem are not yet available (being in progress in our Lab at the present moment): however, we can say that in a creeping oxytricha activation induces periodical, violent, forward jerks, which enable it to engulf any suitable (=of the right size) prey (Ricci & Riggio, unpublished results). Although only very few things can be said about this step in the induction of giants of *O. bifaria*, a sort of working hypothesis can be proposed, to orientate our future research: does activation affect the bioelectrical state of the temporarily predisposed cells? What is the role played by the specific protein(s) synthesized during the LAW in this change of the electrical properties of the membrane? Answers to these two guide-questions will help us in further penetrating the nature of the biological processes constituting that complex phenomenon called induction period.

E The First Giant (FG)

The end of the induction period is represented

by the appearance of the First Giant, namely of that organism easily and unfailingly distinguishable from the other cells for its clearly altered morphology: it must be noted that it is exactly the same as that of the Steady State Giants that will appear later on in the population. The most typical traits of the FG are the strikingly irregular morphology, which is due to the large number of preys engulfed within roundish food vacuoles, together with darker cytoplasm, larger dimensions of the body and of macronuclear pieces as well. Only very recently has it been possible to measure both micro- and macronucleus DNA content. As far as these two very important parameters are concerned the FG definitely presents no significant difference at all from normal cells. This finding cannot but suggest that the first morphological alterations of the giants are induced by the relatively extraordinary diet, the true, nuclear regulation of the process occurring only later.

We must recall here that only a very small percentage (1–2%) of *O. bifaria* can differentiate directly into FG, while most of the giants of a population (>98%) are produced by transverse binary fissions of preexisting giants [59]. An important, still unsolved problem related to the formation of the FG is represented by its widened peristomial area, namely that lying between the AZM and the UM ($\sim 30^\circ$ in width vs the 15° in normal organisms [66]): is it a feature of the temporarily determined cells? Is it acquired during activation? Is it the consequence of the many successive predatory events?

F The binary fission of the FG

When the FG recovers from the phase of dramatic cellular changes undergone to begin its own existence, a binary fission occurs, which gives rise to the first generation of Steady State Giants (SSG): during this division the macro- and micro-nuclear DNA content increases heavily, reaching the quantitative strain-specific values characterizing the SSG. The reason why this value for the macronucleus is ~ 3.7 times the normal value in #C9 [69], and of ~ 1.9 – 2.1 times the normal value in #S9 [6], is still to be ascertained, as well as the origin of the extra-DNA itself: is it synthesized *ex novo*, or does it come from a recycling of that of

the preys?

As to the micronucleus, both #C9 and S9 SSG show the same DNA content, about 1.9 times the normal quantity. The reasons why in different strains different increases of DNA content occur in the macronuclei and not in the micronuclei are not yet understood. The most relevant result, however, is the finding itself; it is a well established topic among ciliatologists that the micronucleus is a sort of unchangeable, diploid genetic memory in each species. That of the *O. bifaria* giant is therefore quite a rare exception to the general rule, and it cannot but deserve to be investigated further, for instance from a cytological point of view: could any chromosomal alteration be detected in comparison with what is described for normal cells [32]?

G The Steady State Giant (SSG)

SSG are those giants regularly undergoing a series of cell cycles, thus reproducing regularly through apparently regular binary fissions: their cell cycle is about twice as long as the normal one. The general kinetics of their growth is described by a logistic curve, where the log period is represented by the *ex novo* dedifferentiation of the few First Giants, the log period is the expression of their intensive binary fissions, while the plateau is reached when the available preys become rarer and scattered through progressively wider spaces [66].

To describe such an interesting heteromorphic form thoroughly, the general morphology has been studied at the cytological [66] and ultrastructural levels [67]. The general shape of a normal *O. bifaria* (the upper half of a rotation ellipsoid) becomes more irregular in the SSG, due to the widening of the peristomial funnel at the anterior end of the giant (already mentioned in the paragraph about the First Giant) and to the preferential accumulation of the food vacuoles in the posterior two thirds of the body, which assumes roughly the shape of half a pear. While all the particulars are described elsewhere [67], three major results deserve particular attention: (i) the normal 8 frontoventral cirri become 10–12 in SSG; (ii) the paraoral external (of the Undulating Membranelles) from a double ciliary array passes to a

triple or multiple ciliary array; (iii) the AZM strikingly increase the number of membranelles, exactly doubling the number of cilia per single membranelle: 3, 16, 22, 22 in normal cells, 3, 32, 44, 44 in a SSG.

These alterations well describe the change of the trophic niche of *O. bifaria*'s SSG, an organism which in fact needs wider peristomial and stronger "predating" organelles, being specialized in feeding on large, sturdy preys. The digestive system of a SSG, generally studied in [66], proved to be particularly interesting, being formed by many vacuoles, each containing either one single prey or a large amount of bacteria, the two foods being never mixed together within the same, single vacuole [67]; it seems likely, that in this way, different enzymatic batteries can be activated around different vacuoles, thus making the two digestive strategies more effective.

The last and perhaps the most important cytological trait of an SSG is its already mentioned higher degree of micro- and macro-nuclear DNA content [60, 69], a biological trait actually characterizing the differentiating process.

Moreover the SSG proved to depend upon continuous active cellular contacts (=bumps) to maintain its differentiated state. The bumps must occur continuously between cortically normal cells: Con-A used separately either on SSG or on preys can interrupt this flux of information about the presence of preys, thus removing the continuous trigger maintaining the gigantic state or, in other words, initiating the process of dedifferentiation. Experimentally to interrupt the normal reproduction cycle of the SSG, it is sufficient also to isolate the giants in cell-free media: this clearly imitates the natural conditions at the end of a ciliate bloom in a certain volume. This shows that the maintenance of the gigantic state is continuously controlled opportunistically by a sort of feedback, capable of promptly revealing the progressive exhaustion of the specific *pabulum* (other ciliates): as soon as this situation occurs, the processes leading a SSG back to the normal state of the species are triggered.

To conclude about the nature of the SSG, we can well state that it represents a reversibly differentiated phase, specifically acquired by the spe-

cies, to exploit a new, predatory niche: specific traits facilitating such a role are developed by this primarily bacterivorous species: (a) wider peristomial funnel, (b) stronger ciliary organelles, and (c) non-oriented (=blindly casual) prey detecting system.

H The Dedifferentiation

In absence of a sufficient number of bumps, a SSG undergoes 4 binary fissions which specifically lead it back to its normal morpho-physiological state. Body size, nuclear size and DNA content have been measured during this process, and found to decrease progressively; in particular, the size and the DNA content of macronuclear apparatus pass from 220 μm and 371 arbitrary units (AU), respectively, to 200 μm and 380 AU after the 1st division, to 160 μm and 257 AU after the 2nd, to 100 μm and 177 AU after the 3rd division, to 102 μm and 108 AU after the 4th: being ~ 100 , the control values of macronuclear size and DNA content, it becomes evident that, while the normal size is acquired after 3 cell divisions, the normal DNA content is established again only after the 4th division. Bacterized medium, autoclaved lettuce medium and SMB (Synthetic Medium for *Blepharisma*, [44]) were used as dedifferentiating conditions: it was found that dedifferentiation occurs according to different kinetics, that in bacterized medium being capable of inducing the fastest dedifferentiation, the second being the autoclaved lettuce medium, the third the SMB [60]. Overall these results seems to indicate that this process clearly depends upon some energetical energy input, but more precise experiments are required before drawing conclusions from this story. We are at present carrying out a series of experimental sessions to draw the ethogram of these ex-giants, to test the general hypothesis [16] about the meaning of the newly "normal" individuals as a sort of exploration shuttles (cf. the *Tetrahymena*'s swimmers), by which the species, after such a differentiation, quickly and efficiently spreads through the environment to find out new possible favourable conditions, where new populations can settle and grow.

II The Protozoa and the "invention" of cell differentiation

First of all, we must recall the mental "Copernican revolution" invoked in the Introduction section: we must keep it clearly in mind that protozoa were the first eukaryotic living entities to colonize the primeval ocean for hundreds of millions of years before the pluricellular organization was reached: "All the pluricellular eukaryotes evolved from unicells, in which the fundamental traits of development appeared" [22]. Such a statement must guide our culture in approaching many biological problems correctly: with regard to "cell differentiation" we must recall that, though representing a typical aspect of the biology of metazoa from a cultural point of view, it is actually one of the brightest "inventions" of protozoa, which used it to face several, dramatic survival problems. The definition itself of development has "developed" over recent years, passing from "the growth from one embrional stage to the next" (typically given for metazoa) to "the progress of an organism through its life cycle", a general concept perfectly fitting the case of protozoa and of *Oxytricha*, as well, once we consider its life cycle as represented in Fig. 2.

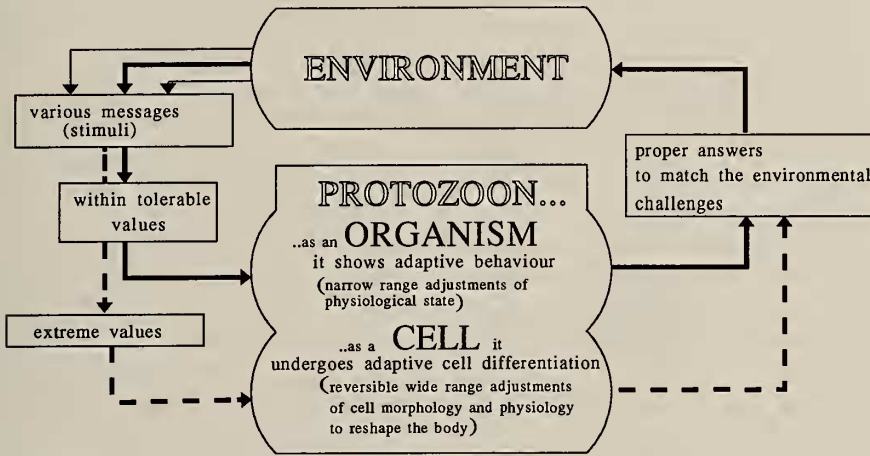
If we proceed in this attempt to establish terminological parallelisms, which in turn underly substantial similarities between protozoa and metazoa, we can consider now the main, three classical steps characterizing the development of a multicellular organism: (i) cell differentiation, i.e. the process leading from one single stem cell to many different types; (ii) pattern formation (the process leading the different types of different cells to congregate in a certain, well defined organ); (iii) morphogenesis, i.e. the mechanical process underlying organism shaping and tissue generation. According to the most commonly given definition of cell differentiation ("cells with one single genotype give rise to definitely different phenotypes"), there can be very little to debate, about the fact that the production of cysts and giants by *O. bifaria* actually are cell differentiations: strikingly different morphological and physiological states are generated, not through any casual abortive process (as has been suggested for the doub-

lets of *O. bifaria*, [2]), but rather through genetically encoded clearcut cytoplasmic reorganization(s) and specific protein synthesis.

This parallel holds further, if we consider that in the cell differentiation of the metazoan developmental processes two basic strategies have been described [28]: (a) long-range interactions mediated by soluble inductors (messages) such as those involved in the metamorphosis of Amphibians [37] and Insects [23, 26]; (b) short-range interactions

(cell-cell contacts), as those described by Jacobson [32] and Muthukkaruppan [45], for mouse lens development, Grobstein [27] for mouse metanephros development, Slavkin & Bringas [74] for odontogenesis, Cutler & Chaudhry [10] for rat submandibular gland development, Lehtonen *et al.* [38] for kidney tubules formation. Quite similar is the case of cell interactions described for protozoa, whose differentiative biology is controlled by soluble factors (*Blepharisma's* preconjugal,

PROTOZOA



METAZOA

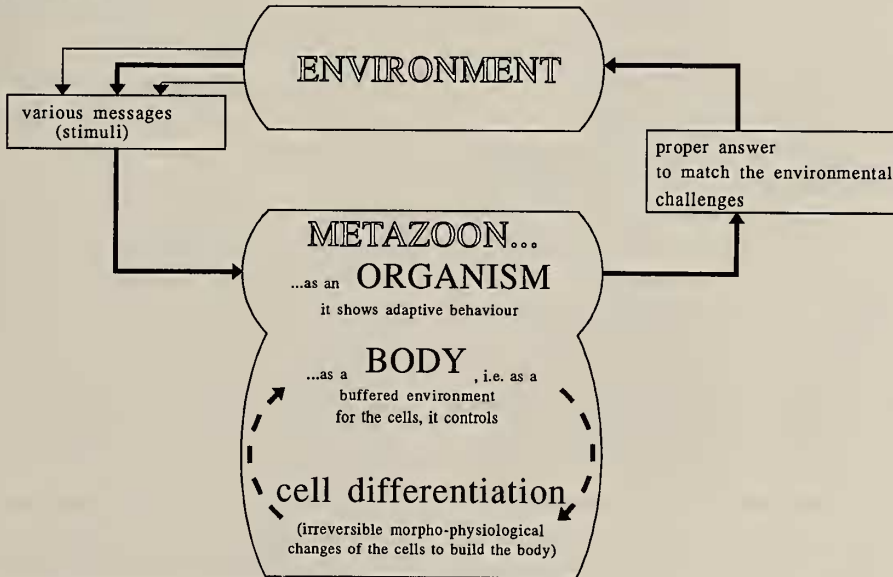


FIG. 4. The parallels and the differences occurring between cell differentiation in Protozoa and Metazoa.

Miyake [44], *Volvox carteri*'s, Kochert [36] and *Dictyostelium discoideum*'s development, O'Day & Lewis [49] or by direct cell-cell contacts (*Chlamydomonas*, Goodenough [24], *Paramecium*'s, Hiwatashi [31]).

Among the other models, *O. bifaria*'s preconjugal story is particularly interesting, inasmuch as the potential partners rely on a peculiar two step interaction process [52]: *O. bifaria* indeed releases the mating type specific soluble factors (gamones) to mediate the first-step cellular interactions between potential partners occurring from a distance [15], while it uses a cell-bound-gamone strategy to guide the partners along the last, critical steps of the interactions leading to membrane fusion, meiosis, pronuclear exchanges etc. [61]: the species, in other words, is capable of using both these different strategies! Why does it use only cell contacts to trigger and to control the differentiation of giants? The opportunistic, highly convenient species-non-specificity of preys discussed above seems to us the evolutionary clue, possibly accounting for such an interactive strategy.

Coming back to the basic problem (can we speak of cell differentiation in the protozoan world?), the classic examples of *Acetabularia* [29, 35, 84] and of *Naegleria* [18] should cancel any doubt. On the other hand most of the efforts spent to convince zoologists working in the field of development that our attempt to make a terminological extrapolation actually represented quite a convenient (and perfectly correct) cultural jump, often came up against two major obstacles.

The first one was that the developmentalists have always said that "Cell differentiation is an irreversible phenomenon among metazoa": in our opinion on the contrary the primigenial cell differentiation on the contrary was a reversible phenomenon (like that found in protozoa), while irreversibility was added only later when multicellularity was reached. There would be no advantage for a protozoon in being capable of skipping environmental stresses by encysting, if it could not resume normal morphology and physiology on the return of favourable conditions. It is obviously true, moreover, that a concept like "irreversibility of the cell differentiation" could not be imagined except for a metazoon, which is a

multicellular organism capable of spending even millions of cells for just one function (electrical conductivity, contractility, distribution, etc.), not needing at all any dedifferentiation! This point of view seems well supported by the finding that whenever such a cellular "expendibility" is obtained by protozoa too, the same irreversibility is also realized: the case of *Volvox* seems paradigmatic!

The second objection to our efforts to establish strong parallelisms between protozoan and metazoan cell differentiation was somehow less critical, on the one hand, and yet more complicated to answer, on the other: the trigger and the control system of cell differentiation is "internal" in Metazoa, but "external" in Protozoa! If we consider the scheme in Fig. 4, the meaning of this observation is perfectly evident: in the case of protozoa, the environment modulates the behaviour of an organism to induce the proper adaptive answers to the environmental changes under normal conditions, while it stimulates cell differentiation under extreme conditions, as a sort of deep morphophysiological adjustment of the entire body to the environmental challenges. In the case of metazoa, on the contrary, the same environment, although acting clearly on the organism at the level of its adaptive behaviour, does not exert any direct effect on the cell differentiation itself, which relies upon specific messages released by another very peculiar and buffered "environment", namely the whole body, which ends up by playing the role of a sort of fairly complex interface between the cells and the external environment.

Before concluding this part of the discussion, we should like to recall also "pattern formation" and "morphogenesis", the other two stages of development after cell differentiation: if one considers the complex life cycle of *V. carteri* [36] and the sophisticated one of *D. discoideum* [49], it seems to us that also as far as these two stages of development are concerned, protozoa show quite complex behaviour and unexpected capabilities. Therefore it seems to us perfectly appropriate and justified to use the biological concepts (not only the terminology) of cell differentiation etc. also when speaking of protozoa.

		<i>Frontonia</i>	<i>Tetrahymena</i>	<i>Blepharisma</i>	<i>Playophryisma</i>	<i>Lembadion</i>	<i>Stentor</i>	<i>Gastrostyla</i>	<i>Oxytricha</i>	<i>Stylonychia hymenostoma</i>	<i>Onychodromus</i>	<i>Oxytricha bifaria</i>	
Inducing conditions	internal (s.l.)	■	+	+	■	(+)	+	■	■	+	■	+	
	external	weak preys	+	■	+	■	■	■	■	■	■	■	-
		starvation	-	-	+	■	+	-	■	+	+	+	-
		high cell density	■	-	■	+	+	■	■	+	(+)	■	+
Small percentage of ex-novo differentiated organisms		+	-	+	■	+	■	■	(+)	+	+	+	
Steady state heteromorphic organism	giant	-	+	+	+	+	-	+	+	+	+	+	
	large n ^o (%)	-	+	+	■	+	■	■	+	+	+	+	
	wide peristome	(-)	+	+	+	+	■	■	■	+	■	+	
	richer ciliature	■	(+)	(+)	+	+	■	■	+	+	■	+	
	larger nuclei	-	+	+	+	+	■	■	■	■	■	+	
	n ^o preys/cell	3	6	6	■	■	■	■	6	11	■	12	
	total digestion	+	■	+	■	+	■	-	+	■	■	+	
adaptation to carnivourism		-	+	-	■	-	■	■	-	+	-	+	
return to the normal state through regulatory divisions		■	+	+	■	+	■	■	■	+	■	+	

FIG. 5. A comparative, synoptic scheme of what reported in bibliography about the reversible cannibalism-gigantism-carnivourism in ciliates; ■ = information ignored by the authors; (+) = reported by the authors as a minor observation; + = yes!; - = no!

III The Protozoa and the “invention” of the “consumers”: new ways of exploiting environmental resources.

There can be no doubt that modern protozoa represent the descendants of the first eukaryotes: they appeared in a primeval ocean completely colonized by prokaryotes, which represented a potentially limitless *pabulum*, for any organism capable of feeding on them [25]. This concept introduces to us the idea that the first protozoa found themselves literally “embedded” in a “full-food” substrate: the primary consumers, seen as new exploiters of the environmental resources, appeared therefore quite early in the evolutionary history of protozoa, which, due to both their “superior”, eukaryotic design and the endless food source (prokaryotes), underwent a tremendous adaptive radiation, concerning them so deeply and dramatically, that the widest morpho-physiological variations on the theme “eukaryotic cell” were realized. Of all the new adaptive solutions one in particular is to be considered in the context of our present paper, namely the conquest of the trophic niche of the secondary consumer, as a consequence of that evergrowing, as yet ungrazed *pabulum* represented by the protozoa themselves. This second step made by the primitive eukaryotic unicells in the recent world of the consumers represented quite an important achievement, because it completed the first food chain ever: deep and extensive studies of the classic examples of protozoan predation [1, 13, 43, 77] might guide us to a more correct comprehension of the phenomenon “carnivorism” in its general lines, in its essential, basic traits. The study of *Litonotus lamella* which predates specifically *Euplotes crassus* [68] led us to show that, although very simple and primitive, this organism had already realized an almost perfect carnivore: *Litonotus*, indeed, has specific systems to recognize and locate its prey (Ricci & Verni, in prep.), to kill it [73], to ingest and to digest it [74]; with respect to the more renowned wolves or lions, *Litonotus* lacks only the social dimension of the hunting pack.

In our opinion, the correct approach to the phenomenon of the reversible cannibalism-giantism-carnivorism (CGC) in protozoa is to con-

sider it as a very peculiar phenomenon, somehow representing an intermediate trophic niche, for the ciliate capable of behaving as herbivour or as carnivour organism. A hypothesis might even be put forward, concerning the possibility that the phenomenon CGC could represent, and somehow testify, that intermediate stage of evolution, when ciliates were neither fully primary consumers nor fully secondary consumers: could the advantage of being an active predator in the proper conditions lead a bacterivorous species to acquire the reversible capability of becoming carnivorous, before becoming a fully irreversible carnivorous species?

We tried to reconsider as many bibliographic items as possible dealing with the CGC study: although a large number of reports is available in the field, it must be stressed, however, that they are only relatively useful in drawing a complex picture of this problem for several reasons: (a) the total number of the ciliate species studied for their CGC is very small, no more than fifteen out of thousands, characterized by extreme diversity and heterogeneity; (b) they belong to widely different systematic groups, so that any possible similarity among them might actually be due to different phylogenetic evolution and/or to different adaptive strategies, thus making it impossible to unify interpretations, in terms of homologies/analogy, possibly accounting for the observed CGC process; (c) the reports range in time from 1853 [30] to 1993 [37]: this extremely wide span makes most of the papers only partially comparable, due to the different minds, cultures, attitudes the researchers had not only toward the problem, but also toward the way of reporting the data: simple, direct, descriptive, naturalistic the earlier (roughly up to the sixties), eminently technical, elaborate and mainly focussed on cell biology aspects, the most recent.

Bearing all these considerations in mind, however, we tried to put the things together, in an attempt to identify, where possible, common features and elements, indicating to us, at least tentatively, the basic fundamentals of the adaptive logic of CGC phenomenon, in order to be able to propose a unifying working hypothesis to guide future research in this field toward more ordered, more comparable and more interpretable results.

The papers we studied deal with *Frontonia* [81],

Tetrahymena [4–6, 14, 42, 73, 83], *Blepharisma* [20, 48, 50, 77], *Platyophryides* [51], *Lembadion* [37], *Stentor* [19, 39, 76], *Gastrostyla* [85], *Oxytricha hymenostoma* [11], *Stylonychia* [21], *Onychodromus* [86], *O. bifaria* [59, 60, 65, 66, 67, 69]. The results just described led us to put together a sort of patchwork (Fig. 5) describing the main characters (horizontal lines) known for the different species (vertical columns). The first consideration is that a very large number (58) of the 154 possibilities are black (=“unknown”), and that 7 more refer to information not reported directly by the Authors, but just found as small, secondary observations. In other words ~40% of the possible, basic information is not yet available even for the few species considered! In spite of all the handicaps so far mentioned, however, a sort of Common Denominator, formed by several basic elements, seems to emerge from the scheme of Fig. 5.

A The induction conditions (internal and external)

Independently from the different single, species-specific solutions, this general character well indicates a basic adaptive value of the CGC phenomena in ciliates: a physiological, internal, very likely epigenetic (*sensu* Nanney, [46]) predisposition is controlled and determined by environmental modulations (proper, weak preys-starvation-high cell densities).

B The number of the ex-novo produced heteromorphic CGC organisms

With the only exception of *Tetrahymena* (which evolved the quite singular stomatin-induction of giants) the other species seem to share the same adaptive strategy: very few heteromorphic organisms differentiated *ex-novo* undergo intense cell binary fissions, for producing massively CGC. This finding seems to represent one of the clearest traits shared by most of the species studied. Why, on the contrary, should they prefer this solution (few changed FG reproduce many heteromorphic SSG instead of the *Tetrahymena*'s strategy (many changed SSG) is quite a complex question, far from being resolved, on the basis of the data so far available.

C The body size

Only *Frontonia* and *Stentor* do not enlarge the body to fulfill the new adaptive tasks, while the other 9 genera produce overdimensional individuals. The sub-characters, namely wider peristomial structure, richer ciliary organelles and larger nuclei seem to follow unfaithfully (automatically) the shift in body size, as possibly confirmed also by the case of *Frontonia*, where normal sized organisms have also normal peristome, ciliature and nuclei. The adaptive meaning of this deep, morphological reshaping seems to be quite clear, once the CGC nature of the heteromorphic phase is considered. It can be easily observed that (a) normal size cannibals (*Frontonia*), (b) gigantic cannibals (*Blepharisma*, *Lembadion*, *Onychodromus*) and (c) gigantic carnivores (*Tetrahymena*, *Oxytricha bifaria*) are described, while (d) no small carnivore has been found so far. If this element should be confirmed by future research, it might represent an important clue to the penetration of the intimate nature of a predator, which it is to be expected should be considerably larger and stronger than its preys, to work as an efficient carnivore. The case of *Frontonia*, in this context, seems to us the clearest example of a purely cannibalistic species, according to three major characters: (i) short lasting tendency to produce cannibals; (ii) small size cannibals; (iii) three preys per cannibal, at maximum.

A final remark must be made with regard to the change in the body shape, when a giant is produced: while all the ciliates seem just to “inflate” their body, regulating them isometrically (=“the giant is just a larger-normal individual), Hypotrichs, on the contrary, seem to have a highly conservative, untouchable portion of their body (namely their ventral surface, with all the locomotory organelles) and an extremely plastic, dorsal one, which, on the contrary, can undergo extensive variations (cf. the *O. bifaria*'s case, as the most paradigmatic in this sense).

D The adaptive meaning

As to the adaptive strategy actually applied by a CGC producing species we must be extremely cautious: many authors, indeed, speak of cannibals

merely because they only experienced that situation in their experiments, without trying to check whether those forms could also be capable of predating non conspecific preys (carnivorous vs cannibal behaviour). According to our data, only the case of *Lembadion's* giant was specifically tested in this sense and found to be a true, cannibalistic form. In general, the two strategies are followed by the different species, roughly in a 1:1 ratio: their basically different meanings have been already discussed in section I and II. To conclude this paragraph which deals with the possible adaptive meaning of these heteromorphic stages in the biology of ciliates, a special word must be said about the *Tetrahymena* example: *T. vorax* specifically undergoes cell differentiation whenever it perceives the substances released by *T. pyriformis*. In our opinion, this seems to indicate that the species has chosen an extremely specialized form of carnivourism, feeding on only one species. This, in turn, cannot but remind us the case of all those species of ciliates specifically predating only one prey (cf. the cases above mentioned). The finding that also among the temporarily, reversibly differentiated predators, a similar strategy has been chosen not only extends the number of those cases, but seems to us moreover to strengthen Fenchel's [16] hypothesis about the significant general advantages of such feeding behaviour, which enables many different species of predators to survive also in one single habitat, without such a wide species diversification.

E *The return to the normal state*

The way by which the different species return to their normal morphology and physiology once the proper CGC conditions are over, namely the series of regulating fissions, represents another apparently univocal solution found by quite different species to solve the same problem. The explanation of the reasons why this solution actually represents such a convenient dedifferentiation path for the species studied could represent a strong contribution to a deeper understanding of the cell differentiation in general.

On the basis of the data already published and of the considerations so far made, we would like to recall how difficult and delicate the correct study of

these phenomena can be even for the most brilliant scientists [9, 87]: therefore it seems to us an absolute necessity, if we extend our knowledge of this theme, to carry on widespread new research, not only to complete what reported in Fig. 5, but also to extend it by adding new species and new elements: only after such a basic investigation phase, will any seriously indicative conclusion become possible.

CONCLUSIVE REMARKS

The data and the arguments above reported demonstrate that, as stated in the first part of this paper, protozoa actually help us in penetrating not only their biology and life philosophy, but also at least some of the most hidden aspects of the general adaptive strategies of Life: their peculiar, unique, double-sided nature, indeed, enables us to put together different fields of Biology, interpreting the same phenomenon according to the ideas and concepts typical of diverse areas. As a simple example, we cite Fig. 6, where the successive steps of the process "*O. bifaria* giant formation" are read as successive phases of a "cell differentiation" and as serial states of an "adaptive strategy", as well.

In other words, Protozoa lend themselves as precious material in biological investigation, representing a solid, polyhedric bridge between cell biology and whole organism adaptive biology: in protozoology, in fact, two quite different and basic aspects of biology (cell vs organism) become directly the two faces of the same coin and, in these conditions, both contribute to define the nature of the coin itself.

In our opinion, this is the most important thesis of the present review.

REFERENCES

- 1 Balbiani EG (1873) Arch Zool Exper 2: 363-394
- 2 Banchetti R, Ricci N (1986) Protistologica 22: 161-168
- 3 Bradbury P, Hausmann K (1993) Ciliates: cells as organisms. Ed. by P Bradbury and K Hausmann, Springer Verlag
- 4 Buhse HEJr (1966) J Protozool 13: 429-435
- 5 Buhse HEJr (1967) J Protozool 14: 608-613

The story of the Giants...	normal culture	over- threshold conditions	induction			giant first	binary fission	steady state giants	back to the normal state
			activation		ingestion				
			E A W	L A W					
...as cell differen- tiation...	not differen- tiated cells	specific trigger	cell-cell contacts	protein synthesis	differen- tiation's accom- plishment	morpho- physiol. changes	nuclear regulation	cell-cell contacts maintain differen- tiation	dediffer- entiation
...as adaptive strategy.	primary consumer	stimulus from environment	physiological preparation	exploitation of a new pabulum	secondary consumer	terminal determi- nation	the preys condition the SSG existence	back to primary consumer	

Fig. 6. The three levels of the story of the giants of *O. bifaria*: the story itself, the cell differentiation, the adaptive strategy.

- 6 Butzel HM, Fisher J (1983) *J Protozool* 30: 247–250
- 7 Cairns JJr (1991) *Environm Auditor* 2: 187–195
- 8 Corliss JO (1979) *The Ciliated Protozoa*. Pergamon Press, Oxford
- 9 Curds CR (1966) *J Protozool* 13: 155–164
- 10 Cutler LS, Chaudhry AP (1973) *Dev Biol* 33: 229–240
- 11 Dawson JA (1919) *J Exp Zool* 29: 473–513
- 12 Dini F, Bracchi P, Luporini P (1975) *Acta Protozool* 14: 59–66
- 13 Dragesco J (1962) *Bull Biol Fr Belg* 96: 123–167
- 14 Dupy-Blanc J, Metenier G (1975) *Protistologica* 11: 159–167
- 15 Esposito F, Ricci N, Nobili R (1976) *J Exp Zool* 197: 275–282
- 16 Fenchel T (1987) *Ecology of Protozoa. The Biology of Free-living Phagotrophic Protists*. Science Tech. Publishers. Madison, WI
- 17 Foissner W (1987) In "Progress in Protistology Vol 2" Ed. by JO Corliss and J Patterson, Biopress Ltd, pp 69–212
- 18 Fulton C, Walsh C (1980) *J Cell Biol* 85: 346–360
- 19 Gelei J (1925) *Arch Protistenkd* 52: 404–417
- 20 Giese AC (1973) *Blepharisma. The Biology of a light-sensitive Protozoan*. Stanford Univ. Press, Stanford, California, pp 123–134
- 21 Giese AC, Alden RH (1938) *J Exp Zool* 78: 117–134
- 22 Gilbert LI (1988) *Developmental Biology*. Sinauer Associates, Inc, Sunderland, Massachusetts, U.S.A., pp 1–635
- 23 Gilbert LI, Goodman W (1981) In "Metamorphosis: a problem in developmental biology" Ed. by LI Gilbert and E Frieden, Plenum, New York, pp 139–176
- 24 Goodenough UW (1977) In "Receptors and recognition Vol 3" Ed. by JL Reissig, Ser. B, Chapman & Hall, London, pp 323–351
- 25 Gould SJ, Raup DM, Sepkoski JJJr, Schopf TJM, Simberloff DS (1977) *Paleobiology* 3: 173–181
- 26 Granger NA, Bollenbacher WE (1981) In "Metamorphosis: a problem in developmental biology" Ed. by LI Gilbert and E Frieden, Plenum, New York, pp 105–138
- 27 Grobstein C (1955) *J Exp Zool* 78: 539–547
- 28 Grobstein C (1956) *Exp Cell Res* 10: 424–440
- 29 Haemmerling J (1934) *Wilhelm Roux Arch Entwicklungsmech Org* 131: 1–81
- 30 Haime J (1853) *Ann Sci Nat Zool* 19: 109–134
- 31 Hiwatashi K (1969) In "*Paramecium*" Ed. by CB Metz and A Monroy, Academic Press, New York, pp 255–293
- 32 Jacobson AG (1966) *Science* 152: 25–34
- 33 Jahn TL, Bovee EC (1967) In "Research in Protozoology" Ed. by TT Chen, Pergamon Press, Oxford, pp 41–200
- 34 Jennings HS (1906) *Behaviour of the Lower Organisms*. Indiana Univ. Press, Bloomington, IN
- 35 Kloppstech K, Schweiger HG (1975) *Differentiation* 4: 115–123
- 36 Kochert G (1975) *Symp Soc Dev Biol* 33: 55–90
- 37 Kuhlmann HW (1993) *Arch Protistenkd* in press

- 38 Lehtonen E, Wartiovaara J, Nordling S, Saxen L (1975) *J Embryol Exp Morphol* 33: 187-203
- 39 Lennartz DC, Bovee EC (1980) *Trans Am Microsc Soc* 99: 310-317
- 40 Lueken W, Krueppel T, Gaertner M (1987) *J Exp Biol* 130: 193-202
- 41 Machemer H (1988) In "*Paramecium*" Ed. by HD Goertz, Springer Verlag, Berlin, pp 185-215
- 42 Metenier G (1981) *Eur J Cell Biol* 24: 252-258
- 43 Miller S (1968) *J Protozool* 15: 313-319
- 44 Miyake A (1968) *Proc Jpn Acad* 44: 837-841
- 45 Muthukkaruppan VR (1965) *J Exp Zool* 159: 269-288
- 46 Nanney DL (1958) *Proc Natl Acad Sci* 44: 712-717
- 47 Nanney DL (1980) *Experimental Ciliatology*. Wiley and Sons, New York, pp 67-75
- 48 Nilsson JR (1967) *Compt Rend Trav Lab Carlsberg* 36: 1-24
- 49 O'Day DH, Lewis KE (1975) *Nature* 254: 431-432
- 50 Padmavathi PB (1961) *Arch Protistenkd* 105: 341-344
- 51 de Puytorac P, Kattar MR, Groliere CA, da Silva Neto I (1992) *J Protozool* 39: 154-159
- 52 Ricci N (1981) In "Sexual interactions in Eukaryotic Microbes" Ed. by PA Horgen and DH O'Day, Academic Press, New York, pp 319-350
- 53 Ricci N (1989) *Limnol Oceanogr* 34: 1089-1097
- 54 Ricci N (1990) *Anim Behav* 40: 1048-1069
- 55 Ricci N (1991) *Mar Pollut Bull* 22: 265-268
- 56 Ricci N (1992) *Proc 3rd Int Congr Neuroethology*, August 9-14, 1992, Montreal, Canada, pp 79-80
- 57 Ricci N (1992) *Acta Protozool* 31: 19-32
- 58 Ricci N, Banchetti R (1981) *Acta Protozool* 20: 153-164
- 59 Ricci N, Banchetti R, Pelamatti R (1989) *Hydrobiologia* 182: 115-120
- 60 Ricci N, Bravi A, Grandini G, Cifarelli D, Gualtieri P, Coltelli P, Banchetti R (1991) *Eur J Protistol* 27: 264-268
- 61 Ricci N, Cetera R, Banchetti R (1980) *J Exp Zool* 211: 171-183
- 62 Ricci N, Erra F, Russo A, Banchetti R (1989) *J Protozool* 36: 567-571
- 63 Ricci N, Erra F, Russo A, Banchetti R (1991) *Limnol Oceanogr* 36: 1178-1188
- 64 Ricci N, Erra F, Russo A, Banchetti R (1992) *J Protozool* 39: 521-525
- 65 Ricci N, Erra F, Russo A, Banchetti R (1991) *Eur J Protistol* 27: 127-133
- 66 Ricci N, Riggio D (1984) *J Exp Zool* 229: 328-337
- 67 Ricci N, Rosati G, Verni F (1985) *Trans Amer Microsc Soc* 104: 70-78
- 68 Ricci N, Verni F (1988) *Can J Zool* 66: 1973-1981
- 69 Riggio D, Banchetti R, Seyfert HM, Ricci N (1987) *Can J Zool* 65: 847-851
- 70 Robinson H, Chaffee S, Galton VA (1977) *Gen Comp Endocrinol* 32: 179-186
- 71 Rosati G, Giari A, Ricci N (1988) *Eur J Protistol* 23: 343-349
- 72 Rosati G, Verni F, Ricci N (1984) *Protistologica* 20: 197-204
- 73 Ryals PE, Smith-Sommerville HE (1985) *J Protozool* 33: 382-387
- 74 Slavkin HC, Bringas PJr (1976) *Dev Biol* 50: 428-442
- 75 Smith HE (1982) *J Protozool* 29: 616-627
- 76 Tartar V (1961) *The Biology of Stentor*, Academic Press, New York, pp 1-413
- 77 Tulchin N, Hirshfield, HI (1962) *J Protozool* 9: 200-203
- 78 Verni F (1985) *Zoomorphol* 105: 333-335
- 79 Verni F, Ricci N (1986) *Symp Biol Hungar* 33: 213-216
- 80 Verni F, Rosati G, Ricci N (1984) *Protistologica* 20: 87-95
- 81 Vimala Devi, R (1964) *J Protozool* 11: 304-307
- 82 Visscher JP (1924) *Biol Bull* 45: 19-27
- 83 Williams NE (1961) *J Protozool* 8: 403-410
- 84 Wilson EB (1896) *The Cell in Developmental Inheritance*, Mac Millan, New York
- 85 Weyer G (1930) *Arch Protistenkd* 71: 139-228
- 86 Wicklow BJ (1988) *J Protozool* 35: 137-141
- 87 Yanbin P, Zuoren Z (1981) *Acta Zool Sinica* 27: 7-11